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BY

CHARLES FRANCIS DE GARIS

A DISSERTATION

Submitted to the Board of University Studies of the Johns
Hopkins University in Conformity with the Requirements
for the Degree of Doctor of Philosophy

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A GENETIC STUDY OF PARAMECIUM CAUDATUM IN PURE LINES THROUGH AN INTERVAL OF EXPERIMENTALLY INDUCED MONSTER FORMA- TION

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FIVE FIGURES

The occurrence of monstrous forms in paramecium has been mentioned by a number of observers, but has been studied in detail by very few. Simpson ('01) found a double-monster "composed of two full-grown Paramecia with organic union" between the parts. This monster lived for seven days, during which time both anterior and posterior components gave rise to daughter-cells of 'good average size.' The experimental production of monsters in paramecium has been effected by Balbiani ('93), Calkins ('11), and Peebles ('12), as the occasional result of transverse cutting at various levels. Such monsters are evidently not comparable to those dealt with in the present report, if one may judge from the following statement of Calkins: "Free cells, complete in all respects, may be given off from the protoplasm of a monster. These may live for days and may even divide, but vitality is weak and they invariably die."

The genetic aspect of the question was introduced by Jennings ('08), who studied a great variety of distorted and monstrous forms in paramecium, especially with the view of observing in subsequent generations the fate of newly acquired structures. In practically all cases he found that such structures were lost through a series of cell divisions, the organisms becoming regulated to typical forms. Stocking

('15) found that after conjugation in paramecium numerous and sometimes very gross abnormalities develop, and that the condition, abnormality, was variously passed on from generation to generation.

STATEMENT OF THE PROBLEM

The question arises for statistical answer: What is the heritable effect of gross abnormality induced not by conjugation, but by some other experimental means? Does monster formation induced in pure lines of paramecium change certain characters which are definitely known beforehand? The present investigation is directed to this question, and the plan of work is outlined in the following propositions:

1. To study a number of pure lines of *Paramecium caudatum* over a sufficiently long period to ascertain, *a*) the fission rate, and, *b*) the mean cell length characterizing each line.

2. To subject a dividing individual of each line to a retarding agent in such a way as to produce a double monster.

3. To study again in pure lines such progeny as may be derived from the monster forms for a sufficiently long time to ascertain, *a*) the fission rate, and, *b*) the mean cell length characterizing the lines so derived.

4. To compare fission rates and cell lengths in each line before and after the interval of monster formation in order to find out whether these measurable characters have been changed by the experience of monster formation.

This investigation is an outcome of work begun in the Zoölogical Laboratory, Johns Hopkins University, in 1924. In connection with this report I take genuine pleasure in expressing my thanks to Prof. H. S. Jennings and Prof. S. O. Mast.

THE EXPERIMENTAL PRODUCTION OF MONSTERS

The point of departure for this work was the development of a method for producing double monsters in paramecium

at will. In many preliminary experiments it was found that exposure of dividing paramecia to the vapors of certain chemicals resulted in delayed division with an occasional double monster persisting after removal of the chemical. The several chemicals used for this purpose were potassium cyanide, ethyl alcohol, formalin, ether, and chloroform. Recently, a wholesale production of paramecium monsters was effected by the simple expedient of placing a depression slide containing a quantity of well-fed paramecia into a moist finger-bowl, the finger-bowl then being covered as a moist chamber and surrounded with cracked ice. The temperature within the moist chamber dropped to 3°C. in less than an hour, and then returned to room temperature as the preparation stood overnight. In this connection it should be recalled, without urging a detailed comparison, that double monsters of *Fundulus* were produced as the result of low temperatures retarding the development of eggs in early cleavage stages (Stockard, '21).

From preliminary experiments it was found that the production of double monsters in paramecium depended on a gradual and fairly prolonged retardation at an early stage of division. Accordingly, the following method was developed: A cell just beginning division was transferred in a drop of hay infusion to a 4 × 4 cm. cover-slip. The cover-slip was then inverted and sealed with vaseline as a hanging-drop preparation on a square-faced watch-glass (capacity 6.5 cc.) containing 1 cc. 1 mol. solution of potassium cyanide. The process of division was soon arrested, obviously by vapor of the cyanide substratum going into solution in the hanging-drop. The cell thus arrested in division was kept under constant observation, and as soon as it began to show signs of deep intoxication (e.g., paralyzed contractile vacuoles, marked increase in thickness, slow ciliary movements, or failing any of these appearances except the arrested division and the equatorial swelling) the cover-slip was removed from the watch-glass of cyanide and sealed over a similar watch-glass of water. By this means the toxic concentration of the hang-

ing-drop was soon reduced, and the cell promptly recovered, but usually remained as a double monster. If cell division did begin again, a second or at most a third exposure over cyanide sufficed to check division completely. The monsters produced by this method all showed thick 'organic union' of their components. Each monster was transferred to hay infusion on a slide depression and cultured by the method to be described below in connection with the treatment of pure lines. The culture fluid was renewed daily, and a free-hand drawing was made of each significant change in form.

Certain statements should be added regarding the cyanide solution. A freshly prepared 1 mol. solution of potassium cyanide was found not to yield as a substratum any toxic vapor to cells in the hanging drop; instead the water of the drop was withdrawn. The same solution on standing well corked one to four days developed a faint, but distinct cyanide odor, and had a marked retarding effect on cell division. Further, since cyanide solutions are notably unstable and since paramecia differ greatly in resistance, it would be misleading to state except within wide limits the period of exposure required for the assurance of monster formation. A few memoranda suffice to show that the effective period of exposure becomes shorter as the age of the cyanide solution increases. Thus monsters resulted from the following exposures: two to three hours over a twenty-four-hour KCN solution; fifty to sixty minutes over a forty-eight-hour KCN solution; fifteen to twenty minutes over a seventy-two-hour KCN solution; signs of deep intoxication developed in less than ten minutes over a ninety-six-hour KCN solution, three exposures being required to stop division. Cyanide solutions older than ninety-six hours were not employed, since their effects were too rapid for control. This rapid action is apparently referable to the presence of ammonia as one of the dissociation products of cyanide. The forty-eight to seventy-two-hour solutions were found most suitable for the present work. In any case, the experiment must be controlled by constant observation of the cell itself, because here as

elsewhere in applying toxic agents to living material no hard and fast statement can be made regarding sublethal and lethal times.

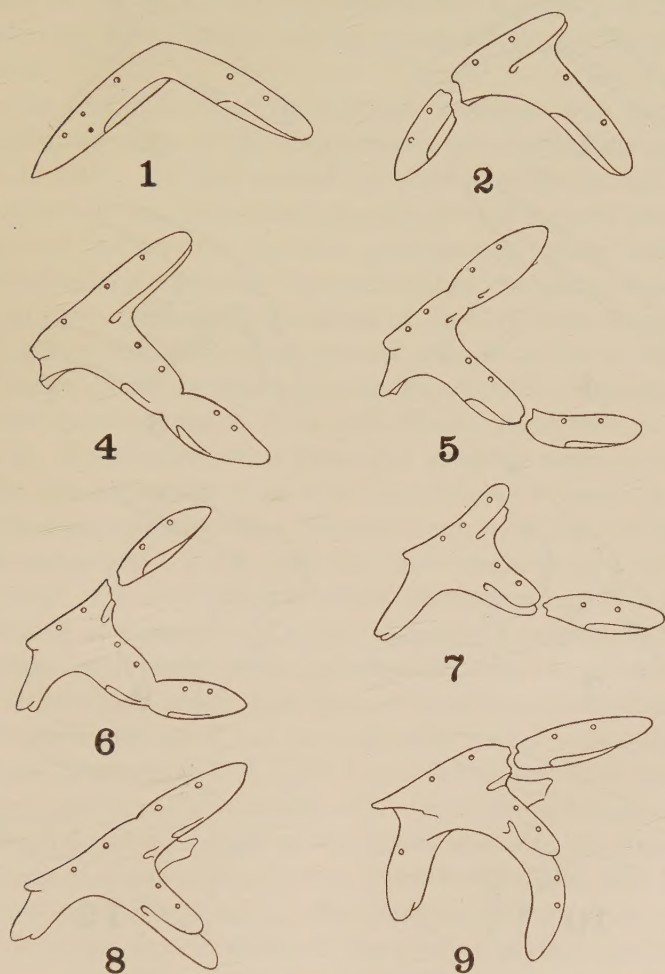


Fig. 1 Representing the sequence of monster 14 during the time it gave off free individuals. The numbers denote age of monster in days.

The monster forms need not be described individually. Figures 1 and 2, from lines 14 and 25, respectively, illustrate in fairly typical sequence the early stages of double monsters.

The late stages shown in these figures are merely samples of residual monsters, of which, as of all the monsters in late

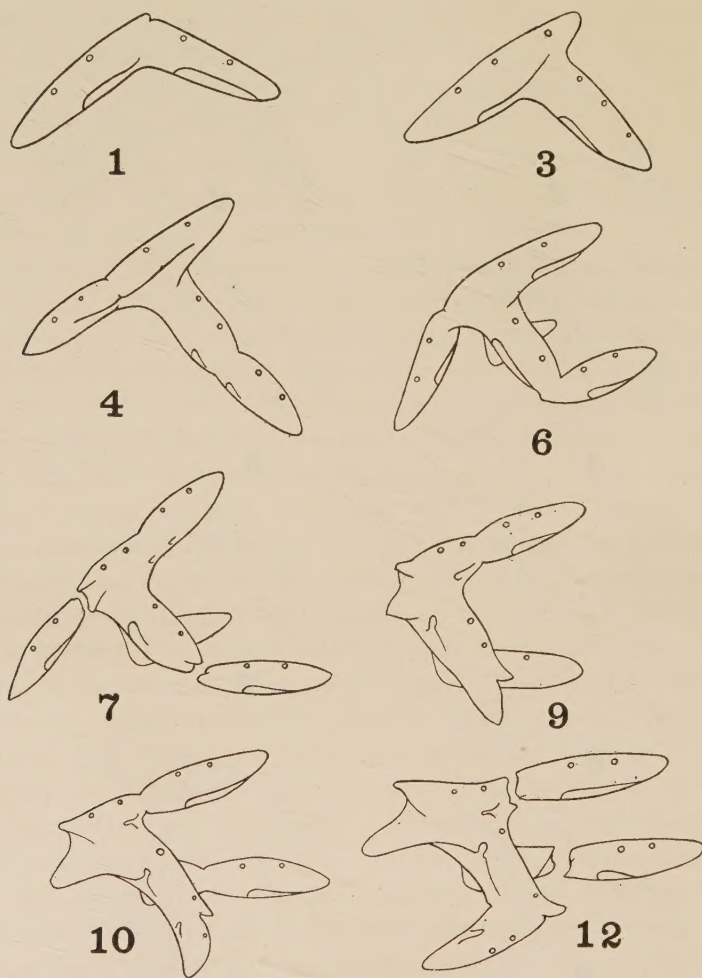


Fig. 2 Representing the sequence of monster 25 during the time it gave off free individuals. The numbers denote age of monster in days.

stages, nothing can be called typical except a general bizarre distortion. Figures 3, 4, and 5 are microphotographs of living monsters. The monster of line 2 during its first day is shown

in figure 3. The monster of line 16 during its second day is shown in figure 4, the overgrowth at the apical end of the posterior component being clearly visible. The residual monster of line 21 during its twelfth day is shown in figure 5, the monster by this time having given off its fourth and last free cell.

During the first day the typical monster was somewhat L-shaped, the axes of the two components enclosing an angle of about 120° . By the second or third day the apical end of the posterior component had usually grown beyond the site of union, and the axes of the two components, when these were not bending or twisting on each other, usually enclosed a right angle. Retarded divisions of one or both components and further inequalities of growth added to the complexity. Eventually, in the typical monster both anterior and posterior components gave rise to free individuals, as shown in figures 1 and 2. Free individuals from the anterior component were usually truncated or distorted posteriorly; those from the posterior component were likewise, but to a less extent, distorted anteriorly. As a rule, the individuals deeply truncated anteriorly did not survive more than three or four days. Something of a departure from this rule is exemplified by the first free individual from monster 14 (fig. 1, second day). This individual, which was deeply truncated anteriorly, did not divide until the third day, when a well-formed posterior cell was produced, along with a very short and distorted anterior cell or cell fragment. This anterior fragment had no oral apparatus, and died on the day following division. The well-formed posterior cell was used to continue the line, as recorded in tables 1 and 2. Residual monsters of the various lines survived five to thirteen days after giving rise to their last progeny. Their failure to survive is in part attributed to malformation or atresia of the oral apparatus.

A peculiar abnormality of the anterior region in *Paramecium aurelia* has been reported by Dawson ('26). He obtained from mass cultures a large number of individuals which were not only truncated, but also invaginated at the

anterior end. Such of these abnormal individuals as were studied in pedigree cultures gave rise to lines characterized by a longitudinal aboral groove and a notched condition of the anterior end, such abnormality persisting through fission, through endomixis, and even through conjugation within the race. From these circumstances Dawson regards the condition as a mutation, and points out that it is the first recorded instance in which a new heritable morphological character has occurred in a ciliate under culture.

It has been my own experience that forms of *Paramecium caudatum*, deeply truncated at the anterior end, may be found rather frequently in mass cultures, especially toward the close of a period of high nutrition, when conjugating pairs prevail, and even more frequently a few days later, along with other unusual forms, some truncated posteriorly, some exceptionally long, some variously bent or twisted. My efforts to continue these unusual forms, as such, in pure lines have met with no success. They either do not survive, or else become regulated to normal types through subsequent division. The work of Stocking ('15), however, is a clear case in point, showing the inheritance of abnormality following conjugation.

No attempt is made to elaborate an explanation of the double monsters produced in *paramecium* by the cyanide method. Only certain general statements are warranted. There are plainly three coincident factors involved in this experimental monster formation: *a*) a retarding agent operating at, *b*) a critical period of development, early cell division, over, *c*) an effective period of time. Attention may be called to one phenomenon, apparently significant in the light of the axial gradient hypothesis (Child, '15), and clearly demonstrated especially in the early stages of these experimental monsters, namely, the rapid growth or overgrowth occurring in the apical region of each component. Further, in connection with the cyanide method, it is interesting to note that Buchanan ('26), studying the effects of hydrocyanic-acid vapor on the developing chick, found in some cases a right-angular branching of the neural tube, which phenomenon he

regards as exemplifying the development of new partial axes, as a result of partial obliteration of the normal anteroposterior axiation. The right-angular and other monster forms produced in paramecium by cyanide vapor could doubtless be described in terms of the gradient concept, but a critical discussion of this question is beyond the scope of the present paper.

THE TREATMENT OF PURE LINES

It remains to state briefly the treatment of pure lines before and after the interval of monster formation. The experiment began with the selection of twenty-eight individuals of *Paramecium caudatum* from four thriving mass cultures. Each organism was cultured on a slide depression in hay infusion. Fresh standard hay infusion was used untreated for the first few days of each week; thereafter it was supplemented by Liebig's beef extract, added in very minute quantity on a teasing needle direct to each slide culture. This treatment was selected, from a number previously tried, as giving the most trustworthy results over a long period.

The work of handling the twenty-eight lines was distributed over two-day intervals, fourteen lines being examined each day. At each examination, 1) a record of the number of generations was made; 2) a specimen was selected for Worchester fixation and measurement, such specimen being neither a small, recently divided cell nor one whose exceptional breadth indicated approaching division; 3) a recently divided cell, if available, or one not obviously approaching division was chosen to continue the line.

The number of generations in eighty-five days preceding monster formation and the mean cell length over the same period for each of the twenty-eight lines are set forth in table 1. Also in second columns of table 1 are set forth the corresponding data for most of the same lines over a period of eighty-five days after monster formation. The lines not so represented are indicated by the terms 'no monster' or 'no progeny.' For instance, in lines 5 and 12, it simply happened

that no cells in beginning division were encountered; therefore, no experimental monsters were produced for these lines. In the other five lines, not represented, monsters were pro-

TABLE 1

Showing, a) the number of generations and, b) the mean cell length in microns for twenty-eight pure lines over periods eighty-five days before and eighty-five days after monster formation

LINES	NUMBER OF GENERATIONS			MEAN CELL LENGTH		
	Before	Monster	After	Before	Monster	After
1	98		100	159.8		158.22
2	107		108	130.14		131.31
3	86		No progeny	176.96		
4	77		81	191.84		190.77
5	105		No monster	135.63		
6	134		128	70.78		70.23
7	69		73	201.54		200.04
8	98		97	169.13		169.67
9	101		99	140.44		141.1
10	94		93	160.33		160.85
11	98		98	151.47		150.9
12	106		No monster	142.55		
13	100		99	164.2		163.87
14	156		159	84.44		84.31
15	95		No progeny	163.33		
16	71		74	198.5		199.13
17	105		105	134.53		135.5
18	98		97	157.74		158.11
19	87		No progeny	174.89		
20	80		No progeny	171.4		
21	115		117	120.1		119.78
22	94		No progeny	158.24		
23	126		126	92.36		93.66
24	94		98	159.09		160.13
25	116		114	121.55		121.7
26	102		105	146.78		147.21
27	88		87	190.89		193.23
28	96		99	162.72		164.45

duced, but the monsters gave off no free cells. It should be understood that each line, as recorded in table 1 after monster formation, was started from the first free daughter-cell of the respective monster.

Since most monsters gave off more than one free cell, it was a matter of interest to collect data as to fission rate and cell length in successive progeny of the same monster. This

TABLE 2

Showing, a) the number of generations and, b) the mean cell length for pure lines derived from successive progeny of five monsters, each record covering a period of eighty-five days after monster formation

LINES	SUCCESSIVE PROGENY	NUMBER OF GENERATIONS	MEAN CELL LENGTH
7	1	73	200.04
	2 a	70	201.3
	2 b	No division	
10	1	93	160.85
	2	92	161.24
	3 a	95	160.35
	3 b	3 divisions	
14	1	159	84.31
	2	157	85.1
	3	154	84.84
	4	158	84.76
	5	154	83.89
23	1	126	93.66
	2	129	92.05
	3 a	125	92.94
	3 b	7 divisions	
	4	No divisions	
25	1 a	114	121.7
	1 b	112	122.23
	2 a	115	120.88
	2 b	112	122.45

work was at first planned to include all progeny of every monster, but such a task soon became overwhelming. As a result, the data recorded in table 2 represent lines continuing for eighty-five days the successive progeny of only five monsters.

RESULTS

Attention may now be called to the results set forth in the tables. In table 1 it is seen by comparing the figures before and after monster formation that the experience of monster formation has produced no significant change either in the fission rate or in the mean cell length for any of the lines. In table 2 it is seen that all surviving lines from the same monster have practically the same fission rate and the same mean cell length. Since the line from the first progeny of each monster represented in table 2 is also represented in table 1, it is seen by comparison of the two tables that there is a close correspondence throughout.

The surviving lines from late successive progeny (e.g., 14 and 25 of table 2 and figs. 1 and 2) exemplify in a most striking way the central facts brought out in this investigation, namely, that the fission rate and the mean cell length characterizing a line are passed on without change through a monster, whose increasing distortion and subsequent death indicate a profound disturbance of cell organization.

CONCLUSIONS

1. A general statement covering the experimental method here used for the production of monsters may be made as follows: double monsters in *Paramecium caudatum* are produced as the coincident result of, *a*) a retarding agent operating at, *b*) a critical period of development, early cell division, over, *c*) an effective period of time.

2. The experimental production of monsters in pure lines of *Paramecium caudatum* does not change, *a*) the fission rate, and, *b*) the mean cell length, characterizing each line.

3. Since fission rate and mean cell length are delicate measures of the genotype, the inheritance of these characters through experimentally produced monsters strongly suggests that the genotype remains unchanged by the experience of monster formation.

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PLATE 1

EXPLANATION OF FIGURES

These figures are microphotographs of living monsters.

3 Monster 2 ($\times 330$) during its first day, the components enclosing an angle of about 120° .

4 Monster 16 ($\times 280$) during its second day, showing an early stage of overgrowth at the apical end of the posterior component. The anterior component is taken in a much extended posture.

5 Monster 21 ($\times 160$) during its twelfth day, after it had given off its fourth and last free cell.



THE EFFECTS OF ANTERIOR AND POSTERIOR SELECTIONS ON FISSION RATE IN PURE LINES OF PARAMECIUM CAUDATUM

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FIVE CHARTS

It was the verdict of Maupas ('88) that among the Protozoa no morphological or physiological differences arise in the products of a given fission or in the continuous generations derived therefrom. Many differences may be found between races or lines of the same species, but within the single race, or 'pure line,' such differences as may arise are not heritable; hence the conclusion that selection for such differences has no effect within a pure line.

Much work could be cited in support of this conclusion. Jennings ('08) attempted to change the size of a race of *Paramecium* by selecting, on the one hand, large individuals, on the other hand, small ones, but his long continued efforts brought out no heritable change in the race. This work was repeated by Ackert ('16) with the same results. Jollos ('13) likewise selected stocks of *Paramecium* differing in resistance to heat and chemicals. He could isolate many races differing in these respects, but within any one race all the individuals were identical. It has recently been shown (De Garis, '27) that an interval of induced monster formation in pure lines of *Paramecium* does not alter the fission rate or the mean cell length characterizing the lines.

As pointed out by Pearl ('17) and by Jennings ('20), the failure to demonstrate heritable diversity within a race offers great difficulty for the theory of evolution. Outside the race

there are many diversities; within the race, apparently none at all. Then how has diversity ever occurred? How is evolution possible? Some say only by sudden changes, by mutations; but, as emphasized by Jennings, these mutations comprise for the most part defects and losses, and it is hard to see how evolution could have progressed by defects and losses. This view, nevertheless, has been maintained by Bateson ('14) and Davenport ('16).

With the stubborn question of race constancy before them, Jennings and his associates undertook further or 'second degree' investigations. The organism chosen by Jennings ('16) was *Diffugia corona*, whose shell presents a number of observable characters. Working with pure lines of these organisms for generation after generation, he selected, on the one hand, those with long spines; on the other hand, those with short spines, likewise those with large shells and those with small shells, those with few teeth and those with many teeth. After long continued selection for these characters, he obtained lines differing from one another in respect to length or number of spines, size of shell, or number of teeth, and, what is very important, these differences were heritable. In short, he found that a single stock, derived by fission from a single cell, gradually broke up into many stocks that were hereditarily different. Other works by the same method and with similar results were carried out by Root ('18) on *Centropyxis aculeata* and by Hegner ('19) on *Arcella dentata*.

One of the first reports of this 'second degree' investigation was the work of Middleton ('15) on *Stylonychia*. Starting with a single individual, he selected, on the one hand, those offspring which divided first; on the other hand, those which divided last. Continuing this selection for hundreds of generations, he found that two sets of descendants were produced from the single parent, one set dividing more rapidly than the other. This difference in fission rate was maintained long after selection stopped.

The foregoing citations bring to a point the leading question of the present work, viz.: Can heritable diversities of

fission rate be demonstrated in pure lines of *Paramecium* by selection on some other basis than that of fission rate itself? Transverse fission in an axiate unicellular organism, as *Paramecium*, gives rise, of course, to anterior and posterior daughter-cells. These are, in the liberal sense of the term, identical twins. Although they are not the mirror images required by Newman ('23), they are to all appearances structurally identical. But are they physiologically identical? Do they give rise to lines having identical fission rates? If the rates are not identical, then is the rate of an anterior line invariably higher than that of a posterior line, or vice versa? The present investigation is directed to these questions.

This work was begun in the Zoölogical Laboratory, Johns Hopkins University, in 1925. For the suggestion of the problem my thanks are due to Prof. H. S. Jennings.

MATERIAL

The organisms used in these studies were individuals of *Paramecium caudatum* isolated from mixed stocks that had been kept in hay infusion over a period of two years (1923-1925). About once a month, these mixed stocks were transferred to fresh hay infusion, although for a period of more than four months during the summer of 1924 no such transfers were made. Dividing organisms were kept almost constantly on hand by transferring large numbers from the stock to 6-cc. watch-glasses and feeding them richly with malted milk. After a few days in the moist-chamber, these watch-glass cultures showed numerous dividing cells. At this time the cultures were in favorable condition for supplying individuals at various stages of division, so that the process could be studied from early prefission stages up to the complete separation of the resulting progeny. By some practice on such material it became possible to select with a degree of certainty the individuals that should be kept under constant observation for early changes preparatory to fission.

The pure lines, to be described below, were cultured in slide depressions and were supplied a varied diet by alternate treatment with the following media: No. 1, standard hay infusion; no. 2, hay infusion plus a small amount of malted milk; no. 3, hay infusion plus Liebig's beef extract. In all the accompanying figures, expressing fission rates, the media used for successive five-day periods are indicated by numbers (i.e., 1, 2, 3, appearing at the top in horizontal order).

It will be noted throughout the figures that medium 2 (malted milk) gave consistently high fission rates, while medium 3 (beef extract) just as consistently gave low fission rates. In subsequent work on pure lines of *Paramecium* with an interval of monster formation, it was found that beef extract added in very minute quantity direct to the slide culture of hay infusion gave much better results than malted milk.

ANTERIOR-POSTERIOR SELECTION

Starting with a dividing *Paramecium*, isolated in a slide depression, the essential difficulty of making an anterior-posterior selection was that of recognizing anterior from posterior progeny, once the two were separated. The only way to do this with certainty was to keep the dividing organism under constant observation, picking out definitely known daughter-cells as soon as division was completed. The dividing organism usually remains quiet during the latter part of the fission process, especially if it is alone in a slide depression. Lacking, therefore, any direction of movement, or any structural features which would differentiate the anterior from the posterior half, one must rely on information obtained before the organism ceases linear movement. A further difficulty arises from the fact that when the daughter-cells do become separated, they frequently remain close together and move over and about each other in such a way as to frustrate the most careful vigil. This latter difficulty was overcome by drawing one or the other daughter-cell aside with a teasing needle and then transferring it to another slide depression.

Five dividing organisms were handled in this way and were used to start five pairs of pure lines. The branches of these lines were further selected for anterior and posterior origin whenever a member was found in division, and in three lines parallel branches were continued as controls. The rates of selected branches and controls within these five lines are shown in figures 1 to 5.¹

In line 1 (fig. 1) within the posterior branch of the anterior stock (*Ant.Post.*) there were twenty posterior selections and a mean fission rate of 1.01, while the control, the anterior branch of the anterior stock (*Ant.Ant.*), was not selected beyond the first instance and had a mean fission rate of 1.17. Here, then, posterior selections in an anterior stock brought out a low fission rate. In the posterior stock there were twenty-five anterior selections within the anterior branch (*Post.Ant.*) and a mean fission rate of 1.62, while the unselected control or posterior branch (*Post.Post.*) had a mean fission rate of 1.16. Here anterior selection in a posterior stock brought out a high fission rate.

In line 2 (fig. 2) within the anterior stock there were six anterior selections and a mean fission rate of 1.65; within the posterior stock there were six posterior selections and a mean fission rate of 1.11. Here, as in line 1, anterior selections brought out the high rate; posterior selections, the low rate, although there were relatively few selections either way.

In line 3 (fig. 3) all branches were selected. Within the posterior branch of the anterior stock (*Ant.Post.*) there were fourteen posterior selections and a mean fission rate of 1.38, while the anterior branch (*Ant.Ant.*), with thirteen anterior selections, had a mean fission rate of 0.90. Within the posterior branch of the posterior stock (*Post.Post.*) there were ten posterior selections and a mean fission rate of 1.12, while

¹ Tables showing the daily fission records of all lines dealt with in this report are on file elsewhere (De Garis, '26). These tables, comprising detailed records from June 5 to December 9, 1925, are much too extensive for publication, but their essential data are available in the graphs of averages presented herewith.

the anterior branch (*Post.Ant.*), with twelve anterior selections, had a mean fission rate of 0.82. In line 3, then, posterior selection in both stocks brought out high rates, while anterior selection brought out low rates, in which respects line 3 is exactly the reverse of lines 1 and 2.

In line 4 (fig. 4) within the anterior branch of the anterior stock (*Ant.Ant.*) there were twenty-one anterior selections and a mean fission rate of 1.36, while the unselected control (*Ant.Post.*) had a mean fission rate of 0.75. Within the posterior branch of the posterior stock (*Post.Post.*) there were nineteen posterior selections and a mean fission rate of 0.60, while the unselected control (*Post.Ant.*) had a mean fission rate of 0.80. Here, as in lines 1 and 2, anterior selection brought out the high rate; posterior selections, the low rate.

In line 5 (fig. 5) within the anterior stock there were ten anterior selections; within the posterior stock, eight posterior selections, and yet the mean fission rates of the two stocks

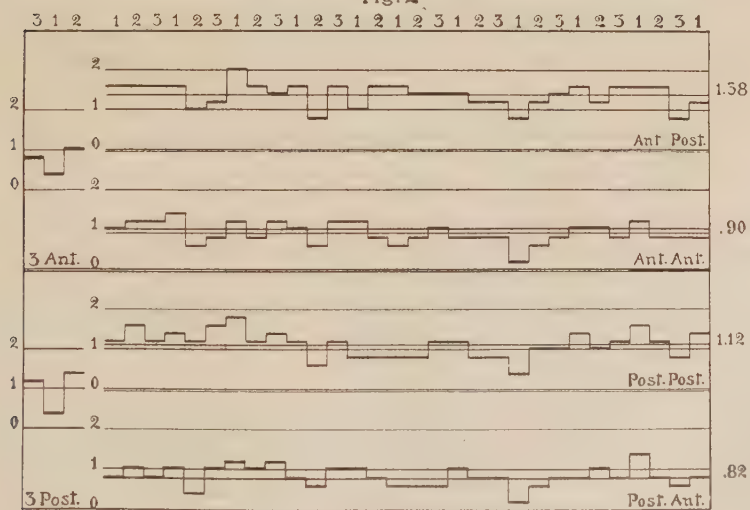
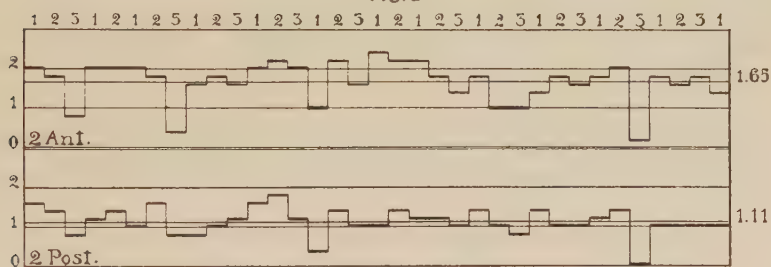
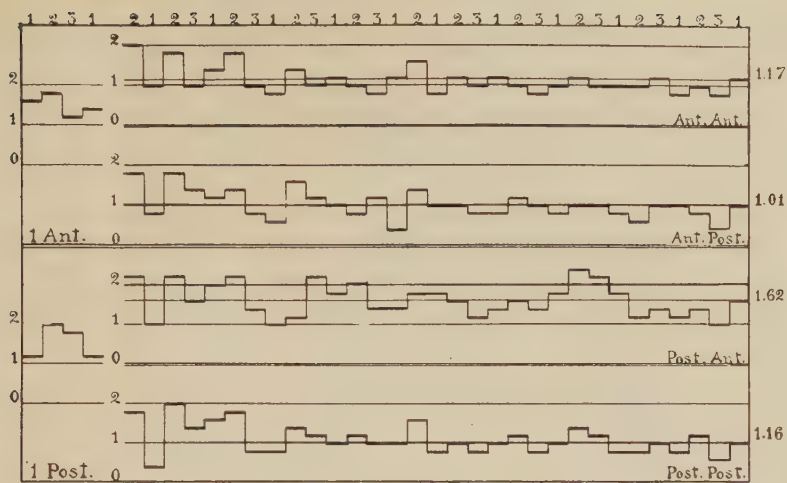
EXPLANATION OF FIGURES

In each of the accompanying figures the average fission rates for successive five-day periods are represented by horizontal bars at or between the levels 0, 1, 2, these numerals in vertical order indicating the range of fissions per day. The line of the arithmetic mean passes horizontally between these levels, and in the right-hand margin the mean rate is expressed to the second decimal. The numerals 1, 2, 3, . . . in horizontal order at the top designate the varieties of culture media used for respective five-day periods: No. 1 is hay infusion; no. 2, hay infusion plus malted milk; no. 3, hay infusion plus Liebig's beef extract.

Fig. 1 Fission rates in line 1. In posterior branch of anterior stock (*Ant.Post.*), twenty posterior selections. In anterior branch of anterior stock (*Ant.Ant.*), no selections. In anterior branch of posterior stock (*Post.Ant.*), twenty-five anterior selections. In posterior branch of posterior stock (*Post.Post.*), no selections.

Fig. 2 Fission rates in line 2. In anterior stock, six anterior selections. In posterior stock, six posterior selections.

Fig. 3 Fission rates in line 3. In posterior branch of anterior stock (*Ant.Post.*), fourteen posterior selections. In anterior branch of anterior stock (*Ant.Ant.*), thirteen anterior selections. In posterior branch of posterior stock (*Post.Post.*), ten posterior selections. In anterior branch of posterior stock (*Post.Ant.*), twelve anterior selections.



were practically identical, that of the anterior being 0.93; that of the posterior, 0.91.

In drawing conclusions from these divergent results, it is well to recall the questions of the investigation: 1) Do the

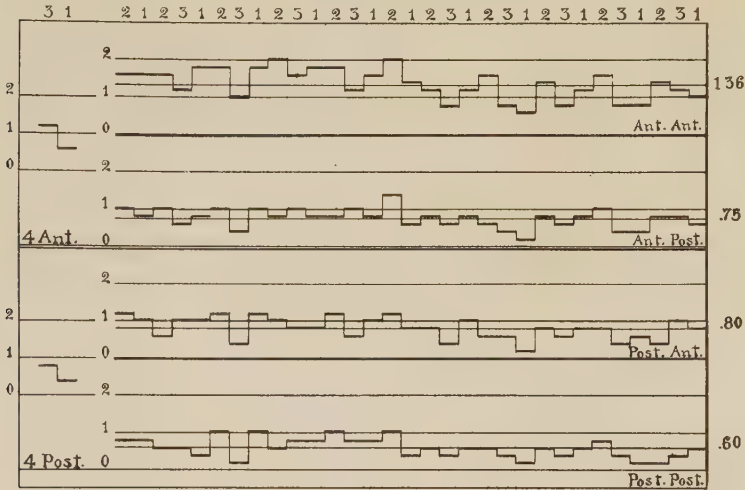


Fig. 4

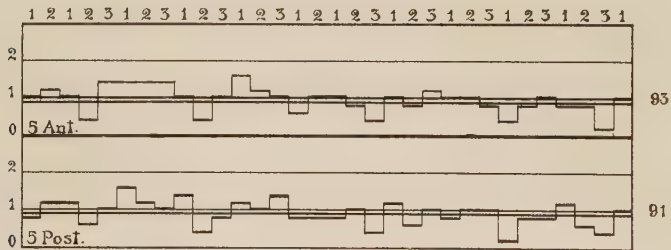


Fig. 5

Fig. 4 Fission rates in line 4. In anterior branch of anterior stock (*Ant. Ant.*), twenty-one anterior selections. In posterior branch of anterior stock (*Ant. Post.*), no selections. In anterior branch of posterior stock (*Post. Ant.*), no selections. In posterior branch of posterior stock (*Post. Post.*), nineteen posterior selections.

Fig. 5 Fission rates in line 5. In anterior stock, ten anterior selections. In posterior stock, eight posterior selections.

two daughter-cells of a single paramecium give rise to lines having the same fission rates? 2) If not, is the fission rate invariably higher for anterior than for posterior lines, or vice versa? The first question may now be answered as fol-

lows: Heritable diversities of fission rate can be brought out in the progeny of two daughter-cells by selecting, on the one hand, cells of anterior origin; on the other hand, cells of posterior origin. Line 5, described above, is the one exception to this statement. The second question must be answered as follows: The fission rates of anterior stocks are not in-

TABLE 1

Showing in anterior and posterior stocks of line 5 the numbers of fissions for successive periods of ten days and the numbers of anterior and posterior selections

ANTERIOR STOCK		POSTERIOR STOCK	
Number of fissions	Number of anterior selections	Number of fissions	Number of posterior selections
11	2	10	
7	1	9	1
14	3	13	2
14	1	11	1
7		9	1
13	1	10	1
11	1	12	1
8	1	8	1
9		9	
7		8	
10		8	
10		9	
6		6	
9		8	
8		9	
6		7	
150	10	146	8

variably higher than those of posterior stocks. In line 3 the higher fission rates are found in the posterior stocks; in line 5 the mean fission rates of anterior and posterior stocks are practically identical.

Since line 5 has been mentioned as an exception to both answers, it is well to present the record in condensed form. Table 1 shows the number of fissions for successive periods

of ten days in the anterior and posterior stocks of line 5, together with the distribution of anterior and posterior selections. It is seen that the fission rates of the two stocks are strikingly similar throughout and bear no significant relation to anterior and posterior selections. While it is the usual experience of those working with Protozoa that one daughter-cell divides before the other, yet occasionally two daughter-cells are found dividing simultaneously. In other words, they are, as special cases, practically identical in respect to fission rate. Line 5 is regarded as comprising special cases of this kind, since individuals in the anterior and posterior stocks were frequently found dividing at the same time. Further, since such anterior and posterior selections as could be made did not serve to bring out an appreciable difference in fission rate, it seems necessary to conclude that no appreciable difference existed.

The answers to the two questions given above and the conclusion regarding line 5 must be set down as the obvious results of the present work. In fact, this work was scarcely designed to give more explicit answers than those obtained. However, certain questions of interpretation should be urged, even if not adequately answered. The foremost questions are these: Is fission rate a heritably fixed character? Or is it constantly changing by small degrees, like the various characters of *Diffflugia* studied by Jennings ('16)?

Since anterior and posterior daughter-cells, as a rule, do not divide at the same time, and since anterior-posterior selection usually results in lines of different rates, the necessary answer to the first question is that fission rate per se is not a heritably fixed character.

Then the first question may be amended thus: Is the time difference between fissions of anterior and posterior daughter-cells a heritably fixed character? To illustrate this point, let r be the particular rate of the parent cell, i.e., the number of fissions per day, and let the difference between $+1$ and -1 be the time difference between fissions of anterior and posterior daughter-cells, where $+1$ merely represents some

unit of increment in rate, and -1 , some unit of decrement in rate. Then does the following sequence hold:

Parent _r	$A_r + 1$	$A_r + 1$ $P_r - 1$	$A_r + 1$ $P_r - 1$	etc.,
	$P_r - 1$	$A_r + 1$ $P_r - 1$	$A_r + 1$ $P_r - 1$	

where any subsequent anterior and posterior daughter-cells have the same difference in rate as the first daughter-cells? Or is there something in the nature of parallel arithmetic progressions, e.g., of increment for anterior cells and decrement for posterior cells, as follows:

Parent _r	$A_r + 0.1$	$A_r + 0.2$ P_r	$A_r + 0.3$ $P_r + 0.1$	etc.,
	$P_r - 0.1$	A_r $P_r - 0.2$	$A_r - 0.1$ $P_r - 0.3$	

where each anterior cell differs in rate from its immediate parent by a fraction $+0.1$, and each posterior cell differs in rate from its immediate parent by a fraction -0.1 ? This is a purely schematic device to illustrate the alternative of a gradually widening difference between anterior and posterior lines.

The two alternatives may be stated briefly in the following questions: Does fission rate change once by a large degree, or gradually by small degrees? According to most of the graphs shown above, the initial difference in rate is almost as great as the difference of the arithmetic means, which apparently indicates that there is a single large change. Yet not only is it difficult to see why the change should occur just once and in the first instance, but also the graphs may be misleading in this respect, since they are averages of five-day periods, during each of which, as a rule, a number of selections were made. It is true, there is no evidence of a gradually widening difference in rate between anterior and posterior lines, but this could be taken to mean that the limits of increment and decrement are not very wide, in which case

relatively few selections would suffice to cover the range of difference (e.g., line 2).²

The present work, however, does not adequately test the alternatives mentioned above. In the first place, selections were not sufficiently successive; in the second place, the time differences of fissions were not noted in hours and minutes, as would be required for a decisive answer. This work, nevertheless, does show that the direction of change in rate is heritable (e.g., if a direction of increment is associated with an anterior stock, it remains so associated, as long as the stock is studied).

The physiological processes underlying these differences in fission rate might well belong to sequences of the ultra-microscopic realm, where, according to Prof. R. S. Lillie ('27), "events are determined by quantum relations or by 'chance' fluctuations of molecular movement." Yet the fact that there is something of the classical macroscopic determination, or quantitative specification of conditions, about these differences has recently been shown by Petersen ('27), who found that at 26° to 30°C. the anterior cell always divides first, while at 13° to 17°C. the posterior cell always divides first. Petersen interprets these findings in terms of the axial gradient concept.

The bearing of the present work on the case of evolution has been sufficiently indicated by the introductory citation of literature and the formulation of questions. The results in this connection may be summarized as conclusions.

² As this communication goes to press an advance abstract announces the paper by R. C. Parker, entitled, "The effect of selection in pedigree lines of infusoria," this to appear in the *Journal of Experimental Zoölogy*. Evidence is promised warranting the conclusion that in Infusoria certain processes of nuclear reorganization (e.g., endomixis) afford proper conditions for the altering of racial characters by selection. My figures (vide supra) show very definite rhythms of depressed fission rate, doubtless indicating endomixis, yet there is nothing in the daily record of fissions to suggest that the rates fluctuate in special relation to these periods of depression. It is a matter of great interest, however, that Parker found "no relationship between the number of selections practiced at any given time and the effect produced." This accords with my own experience, as witness the results of but six selections either way in line 2, compared to the results of three or four times as many selections in other lines.

CONCLUSIONS

1. Heritable diversities of fission rate in pure lines of *Paramecium caudatum* can be brought out by selecting, on the one hand, cells of anterior origin; on the other hand, cells of posterior origin.

2. The fission rate is sometimes higher in stocks of anterior selection, sometimes in stocks of posterior selection.

3. The direction of change in fission rate is heritable (e.g., if a direction of increment is associated with an anterior stock, it remains so associated).

4. From the foregoing statements it follows that fission rate per se cannot be regarded as a heritably fixed character.

5. As a special case, a single line may comprise stocks in which anterior and posterior selections bring out no appreciable difference in fission rate.

6. The fact that diversities of fission rate are associated with anterior and posterior progeny of a single cell is taken to exemplify one means by which physiological diversities arise in the course of evolution.

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